Antibody Binding to Native Whole Cells

Label-free detection and measurement of antibody binding to membrane proteins on intact mammalian cells

Current label-free biosensing techniques like SPR cannot use whole cells. This prevents the ability to look at ligand binding to an important class of proteins – membrane proteins. Instead SPR is only able to work with soluble, proteins in clean buffer.

The goal is to develop and validate antibodies against membrane-bound receptors.

Now for the first time, a biosensor is available for label-free immunoassay on whole cells. It uses the sam®5´s mass response (phase), which predominantly is unaffected by matrix effects.

Setup

In this application note, the antigens are whole breast cancer cells serving as the ligands on the sensor chip. Surface proteins embedded in the cellular plasma membrane with extracellular domains were used for verification of the bound cell by specific antibodies.

Whole cells were captured by dimerization with E-Cadherin monomers. Cadherins are Ca^{2+}-dependent transmembrane adhesion proteins forming and stabilizing cell contacts in different tissues.

Materials and Methods

Measurements were performed with a standard sam®5 biosensor using surface acoustic waves, equipped with gold chips functionalized with –COOH SAM (see previous Application Note 07).

A rhE-Cadherin/Fc Chimera (recombinant human) was bound via carbodiimide chemistry in EDTA containing buffer (conserving the monomers at immobilization) to 2.5ng/cm^2 and 28fmol/cm^2.

Running buffer was then exchanged to PBS + Mg^{2+} + Ca^{2+} to enable E-cadherin dimerization for capture and binding of mobile MCF-7 cancer cells *(see Figure 1)*.

The bound cancer cells were verified using antibodies against different surface antigens at dilution of 1:100:

- E-Cadherin, is a cadherin presented by epithelial cells with a MW of 87.7kDa.
- Ep-CAM (=EPG40), epithelial Cell Adhesion Molecule, a 40kDa transmembrane epithelial glycoprotein.
- EMA, Epithelial Membrane Antigen, a transmembrane protein with 265-400kDa.

The chip surface was regenerated using buffer supplied with 500mM EDTA

Results

Complete MCF7 cells were bound via dimerization to the E-cadherin monomers presented on the sam®5 chip surface *(Figure 2)*. The injected antibodies bound according to quantity and distribution of the different cell surface proteins presented by the cells:

(1) EMA is a heterogenous group of heavily glycosylated proteins expressed by a wide range of tumors;

(2) Ep-CAM is a type I membrane protein frequently expressed by epithelial cells and many carcinomas. Due to its high abundance, it showed highest response at antibody injection;

(3) E-Cadherin, the adhesion proteins.

Antibody binding was referenced by injections without cells.

Kinetic data was evaluated and rate constants calculated using Origin software *(Figure 3)*.

At injection of cells and of the glycerol-containing antibody solution, extremely little buffer effect was visible.

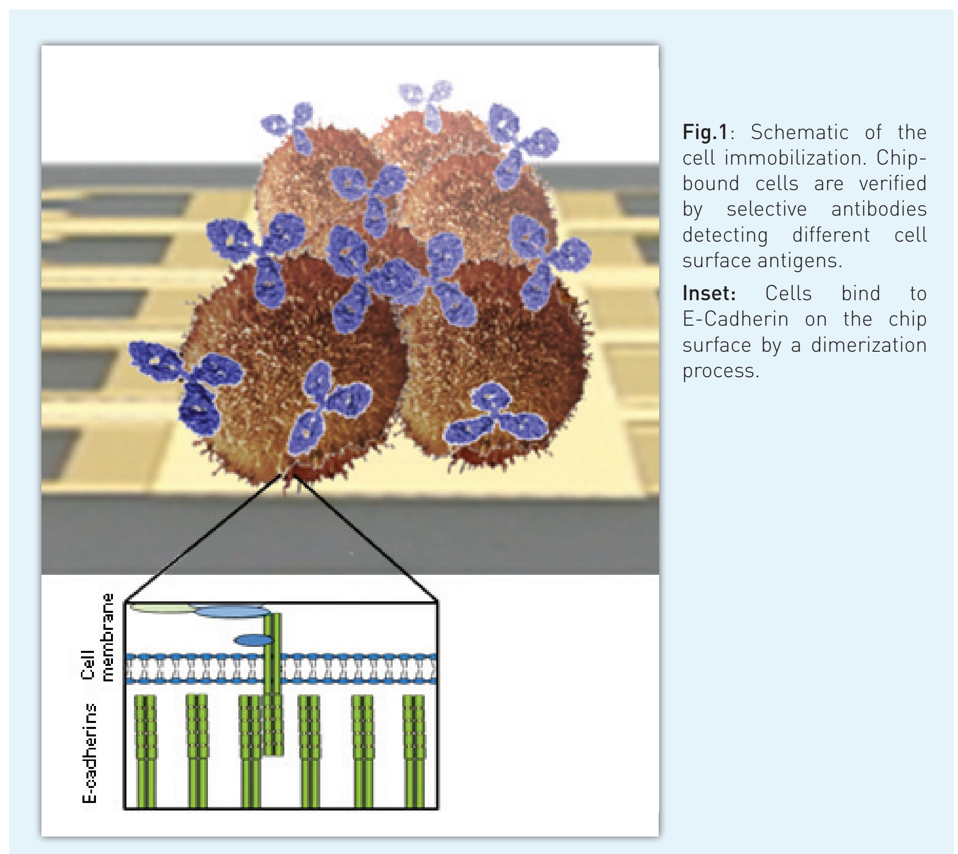


Fig.1: Schematic of the cell immobilization. Chip-bound cells are verified by selective antibodies detecting different cell surface antigens.

Inset: Cells bind to E-Cadherin on the chip surface by a dimerization process.

Conclusion

Cell surface proteins were recognized in their native environment. The antibodies bound the protein targets presented by the cells according to both abundance and affinity. Binding measurements were performed on the chip surface and in a challenging biological matrix using the phase signal.

This is an application which is not possible using SPR. Using the surface acoustic wave system, sam®5, it is simple to perform and a valuable addition to the armory for companies developing antibodies against or targeting towards membrane receptors.

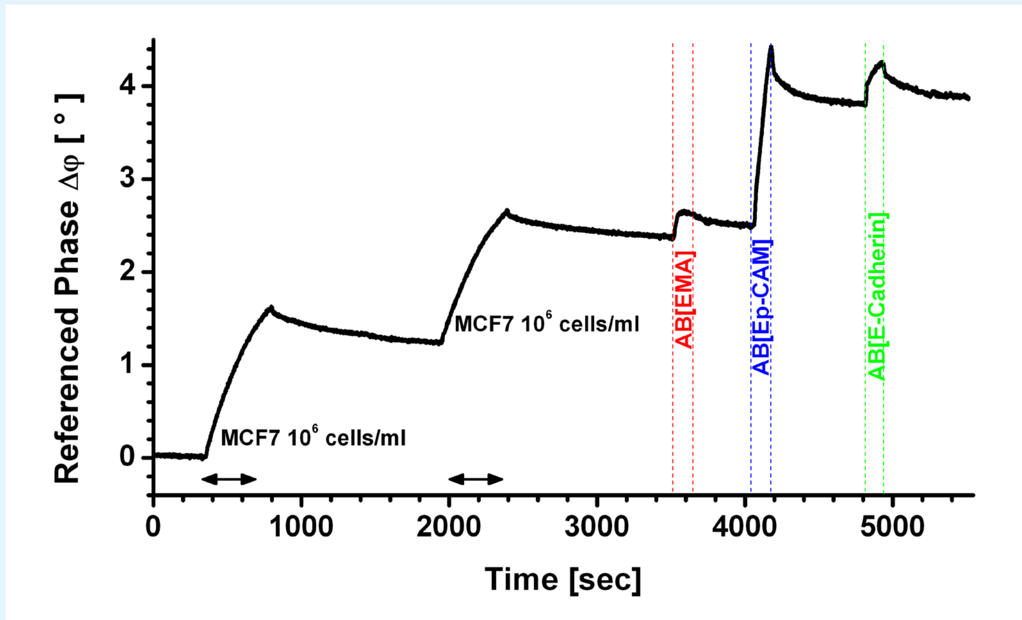
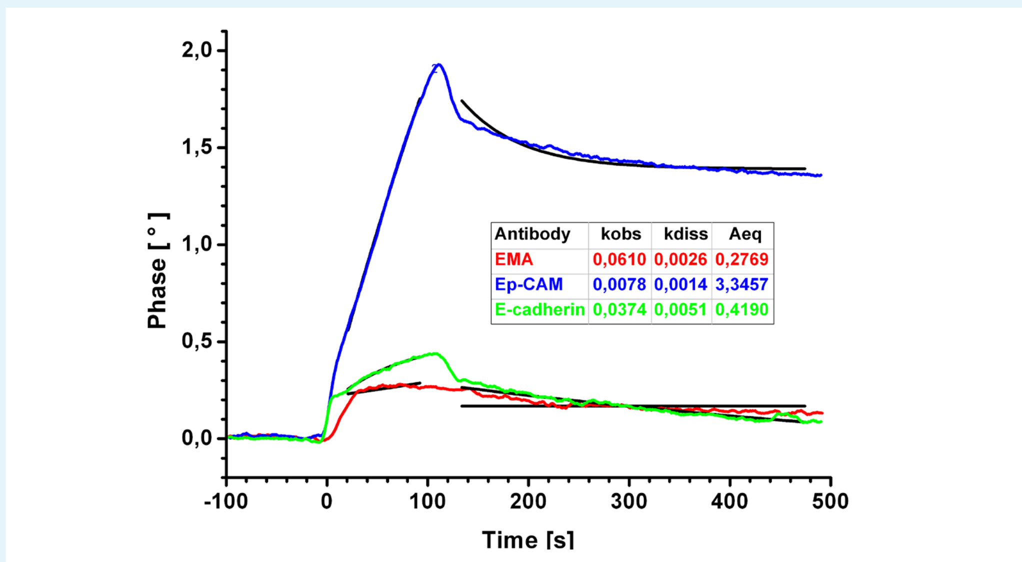


Fig.2: Binding of MCF7 cells and different antibodies recognizing cell surface proteins. The phase response was referenced with a set of antibody injections without cells. Antibodies were injected at dilution 1:100, detecting EMA, Ep-CAM and E-Cadherin.



Antibody	kobs	kdiss	Aeq
EMA	0,0610	0,0026	0,2769
Ep-CAM	0,0078	0,0014	3,3457
E-cadherin	0,0374	0,0051	0,4190

Fig.3: Overlay plot of injections shown. Fits based on a 1:1 binding model were applied and kinetic data evaluated as far as possible, since exact original antibody concentrations were unknown.

saw INSTRUMENTS®

SAW Instruments GmbH
Schwertberger Str. 16
D-53177 Bonn, Germany

Tel: +49 (0) 228 812876-0
Fax: +49 (0) 228 812876-20

email: info@saw-instruments.de
www.saw-instruments.de